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DEVELOPMENT OF A PREDICTIVE SELECTION TECHNIQUE TO IDENTIFY CANOLA SEED THAT LOSE GERMINABILITY AND SEEDLING VIGOUR DURING STORAGE

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Development of a Predictive Selection Technique to Identify Canola Seed that Lose Germinability and Seedling Vigor During Storage

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Development of a Predictive Selection Technique to Identify Canola Seed that Lose Germinability and Seedling Vigor During Storage

(a) Summary

Sixteen cultivars of canola known to vary in seed germination vigour were analyzed by RT-PCR to determine if there was a correlation between aquaporin expression and germination vigour. Gene specific primer pairs were used to detect m-RNA expression corresponding to each of five different aquaporin genes by RT-PCR analysis. The five aquaporin genes selected for this study were P1P1, P1P2, alpha-TIP, delta-TIP and gamma-TIP. Following an exhaustive study, we could not establish a strong correlation between the expression of five aquaporin genes and germination vigour.

Seventeen canola varieties varying in seedling vigour were screened by RAPD analysis to determine if there was any polymorphism linked to germination vigour. Over 500 random primers were used to screen DNA extracted from the seventeen cultivars. Twenty primers revealed polymorphism for seed germination vigour. One primer, UBC317 was able to distinguish nine varieties for low germination vigour and eight high germination vigour varieties. A band at 1300 bp was highly correlated with germination vigour and may be suitable to identify superior genotypes.

(b) Executive Summary

Seed germination is controlled by many genes which makes this phenomena a complex character to study. Over 1000 genes are involved and many of the gene products associated with germination are produced in the seed fusing maturation. These gene products are activated by water uptake. In addition, new proteins are produced by upregulated genes in response to water phytohormones and environmental conditions.

Environmental conditions during seed production have a tremendous impact on the quality and vigour of seeds. Recent research has established seed from fall sown canola has superior germination vigour compared to seed from late May sown canola. The primary reason appears to be the environmental conditions during ripening. Fall sown canola flowers in early June and the seeds are produced under cooler and moister conditions than plants flowering in July. Seed that has high germination vigour tends to produce vigorous seedling that have high synchrony of emergence, well established root system, vigorously out competes weeds, prevents soil erosion and have high seed yields.

One of the main factors controlling seed germination is active water uptake by the seed. Water uptake falls into three phases. The first phase is passive uptake due to a difference in water potential of the seed and the soil. The second phase varies with soil moisture, soil temperature and dormancy of the seed. The third phase involves active water uptake by a group of protein called aquaporins. Aqua means water and porins stands for pores in the seeds membranes. There are two classes of aquaporins; TIPs and PIPs. TIPs are located in the tonoplast and PIPs are found in the plasma membrane. Our initial work established a high correlation between TIPs and PIPs and seed germination vigour.

RAPD's are used to screen DNA for polymorphism for a selected trait. This approach can be used to screen populations for a particular trait and then this trait can be followed by a breeder to select for desirable traits. Since RAPD analysis uses DNA the results obtained are not influenced by environmental factors.

Both of the above approaches were used to develop a screen to select canola varieties that have the potential of high germination vigour. After an exhaustive analysis we could not establish unequivocally that the TIP's and PIP's we analyzed for are associated with seed vigour.

There are over 30 members in both the TIP and PIP family. To develop antibodies to all of them was beyond the time frame and budget allotted to this project.

For RAPD analysis we screened over 500 random primers to determine if we could select varieties of canola that have high germination vigour. Twenty primers revealed there was polymorphism linked to germination vigour. One primer, UBC317, was able to distinguish high germination vigour varieties. In future this band will have to be isolated and cloned for the breeder to use this as a marker in a breeding program. We are quite excited about this finding.

Our previous research established that varieties that germinate quickly (one to two days) at room temperature can be used as a selection screen for superior seed lots. Seed lots that germinate quickly at 8°C can also be used as a selection method. We found that large, dense, round and black seeds (which can easily be identified with a microscope) produce seedlings of high vigour. Thus we have established both simple and somewhat complex tests for selecting seeds.

(c) Technical Report

Germination tests

Seventeen canola varieties obtained from Dr. R. Elliot at AAFC, Saskatoon were identified by Dr. G. Thompson (see previous reports) to range in germination vigour. Dr. Thompson established that seeds with a high germination rate after two days at 21°C were highly correlated with seed vigour tests conducted in the field at several locations. Therefore this test was used as our standard in developing the RT-PCR and RAPD screening tests. In addition we also obtained seed varying in germination vigour that we obtained from 20/20 Seed Labs Inc., Niskw, Alberta. To preserve the identities of the varieties, numbers were assigned to these seed lines.

RNA isolation and RT-PCR

Two grams of seeds from each variety were ground to fine powder in liquid nitrogen. The powder was homogenized with 5 ml of RNA extraction solution (8M Guanidine hydrochloride, 20 mM MES, 20 mM EDTA, 0.1 M sodium acetate, 100 mM mercaptoethanol) and centrifuged at 7000 rpm for 20 minutes at 4°C. The supernatant was extracted with 5ml of chloroform and phenol (1:1), and centrifuged as above. The aqueous phase was re-extracted with the chloroform and IAA (24:1). After centrifuging, the total RNA was precipitated with 100% ethanol followed by wash 3-5 times with 2 M LiCl and once with 70% ethanol. The pellet was dissolved in 50 ul DEPC treated water.

Total RNA (2 ug) was used to synthesized the first strand cDNA with Superscript II reverse transcriptase. Gene specific primers designed for aquaporin genes were used in PCR reactions to amplify cDNA. The PCR products were separated by 1% agarose gel. The DNA bands were visualized by ethidium bromide staining.

DNA extraction and PCR reaction for RAPD

About 1 gram each weight from 5-7 day old seedlings were harvested. The samples were finely ground in liquid nitrogen, and homogenized with 5ml of 2 X CTAB extract buffer (2% CTAB, 1% PVP, 100mM Tris pH 8.0, 20 mM EDTA pH 8.0, 2.8 M NaCl) at 65°C. The samples were incubated at 65°C, in a water bath for 15 minutes and extracted with the same volume of chloroform 24:1 IAA solution, centrifuged in 3000 rpm at 4°C for 10 minutes. The supernatant was transferred to a new centrifuge tube and mixed with 1/10 volume of 10% CTAB buffer (10% CTAB, 1.4 M NaCl). This mixture was incubated for 10 min at 65°C, extracted, and centrifuged as described above. The DNA was precipitated with 100% ethanol, centrifuged and washed with 70% ethanol. The air-dry pellet was re-suspended in 1 ml TE buffer.

Equal amounts of DNA extracted from 5 varieties of low germination vigour were bulked together. Similarly, DNA from 5 varieties with high germination vigour was also bulked together. The two bulked DNA samples were used as the template for RAPD to screen random primers. When a primer showed polymorphism, it was used to screen the individual varieties. PCR was carried out in 25 ul of reaction solution containing 2.5 ul of 10X PCR buffer, 1 ul of 50mM $MgCl_2$, 0.25 ul of 10 mM dNTPs, 3ul of 600 nM primer, 0.25 ul of Taq polymorase (5U/ul), 10ul of template DNA (200ng/ul). The reactions were processed through 35 cycles at 94°C for 1 minute, 37°C for 1 minute, 72°C for 1.5 minutes. The amplification products were separated by 1.5% agrose gel and the DNA bands were visualized by ethidium bromide staining.

Results

Seed vigour and germination

Generally, percent germination during the first 2 days was positively correlated to the final germination percentage (Table 1). The 17 varieties were divided into 2 groups based on their germination rates for the first two days. If germination was over 50% on day 2, the variety was rated as having high germination vigour. Varieties with less than 50% germination on day 2 were rated as low germination vigour (Table 1). Eight varieties (52584, 52588, 52673, 53280, 53595, 53534, 53538 and 53554) had low vigour.

Table 1 Germiñation rate of 17 canola varieties (%)

Time (days)												
Variety Number*	1	2	3	4	5	6	7	8	9	10	11	Rate
52568	0	0	2	2	6	6	7	7	10	16	21	Low
62572	2	31	47	60	67	74	84	87	91			Low
52576	0	0	4	6	7	8	12	14	15	16		Low
52584	54	95	98	99								High
52588	58	85	89	96	98							High
52673	60	82	90	94	96							High
53256	0	27	47	62	67	74	80	87				Low
53280	52	85	88	91	93							High
53595	20	55	69	79	81	83	87					High
53508	0	4	9	15	23	39	45	53	57			Low
53522	0	0	5	11	19	35	41	49	53			Low
53534	42	81	97	2	99							High
53538	48	71	83	86	89	95						High
53542	0	2	4	18	30	43	58	63	74	85	90	Low
53546	1	34	49	65	75	80	85	85	87	87	88	Low
53554	31	73	75	86	91	97						High
67023	2	32	48	54	61	67	72					Low

*The given name of the variety was not known. This was the number given by 20/20.

RT-PCR band pattern was not related to seed germination

Five pairs of gene specific primers (PIPI, PIP2, alpha-TIP, delta-TIP and gamma-TIP) were used for RT-PCR to screen varieties of canola that varied in seed germination vigour. RT-PCR did not detect any significant differences in either TIP or PIP expression in seeds that varied in germination vigour (Fig. 1). Although we screened varieties with six aquaporin primers, the results for only 4 varieties and two primers are presented in Fig. 1.

RAPD band pattern related to seed viability

More than 500 random primers were produced for this RAPD study. Twenty primers showed polymorphism between bulked DNA extracted from seed of high germination vigour and from seed of low germination vigour. One primer coined UBC317 was able to distinguish 9 low seed vigour varieties from 8 high seed vigour varieties. The band pattern amplified by primer UBC317 is presented in Figure 2. A band with approximately 1300 base-pairs (indicated by an arrow in Figure 2) was present in the DNA from the bulk 2 (high germination bulk) and varieties that demonstrated high germination vigour. This band was not present in the bulk 1 (poor germination bulk) and the varieties with low germination vigour.

Discussion

Using RT-PCR analysis, we screened 17 varieties of canola that were previously catalogued by Dr. G. Thompson to have either high or low germination vigour. This was part of a collection given to us by Dr. R. Elliot of AAFC, Saskatoon. Five pairs of gene primers for PIPI, PIP2, alpha-TIP, delta-TIP and gamma TIP were used in the RT-PCR analysis. Unfortunately, after much repeated and extensive analysis, we could not establish a strong correlation between these primers and seed germination vigour. Therefore the approach was abandoned although our initial studies showed high promise that primers could distinguish differences in varieties.

Over 500 primers were analysed for RAPD markers to determine if there was polymorphism for seed germination vigour. Seventeen varieties were obtained from 20/20 Seeds Inc. that were catalogued by this company to be of high and low seed germination vigour. DNA was extracted from all seventeen varieties and then bulked into two pools depending upon what category they fit. Previous tests confirmed varieties that had over 50% germination after two days could be classified as high germination vigour. We used these criteria to produce two bulked samples of DNA for our initial screening of over 500 primers. Of the 500 primers, 20 showed polymorphism for seed germination vigour. Of the twenty, one primer we called UBC317 was able to distinguish individual varieties for high and low seed vigour. The results of this primer are given in figure 2. A band of approximately 1300 base pairs (as indicated by the arrow in figure 2) was present in 8 varieties that were classified as high germination vigour. This band was absent in 9 varieties classified as low germination vigour.

Seed germination vigour is a complex genetically inherited character. In addition, the environment has a profound effect on seed vigour. Small, shrivelled, green seed does not have the same germination vigour as large, dense and mature seeds. Thus the same variety may vary tremendously depending on the environment the mother plant is exposed to. However, in this study we used RAPD analysis to select varieties that have high germination vigour. We are excited and encouraged by these results, because the breeder is now able to select promising canola lines within a segregating population. Future research is required to establish if this primer can be used to select promising lines of our other crops e.g. flax, wheat, etc.

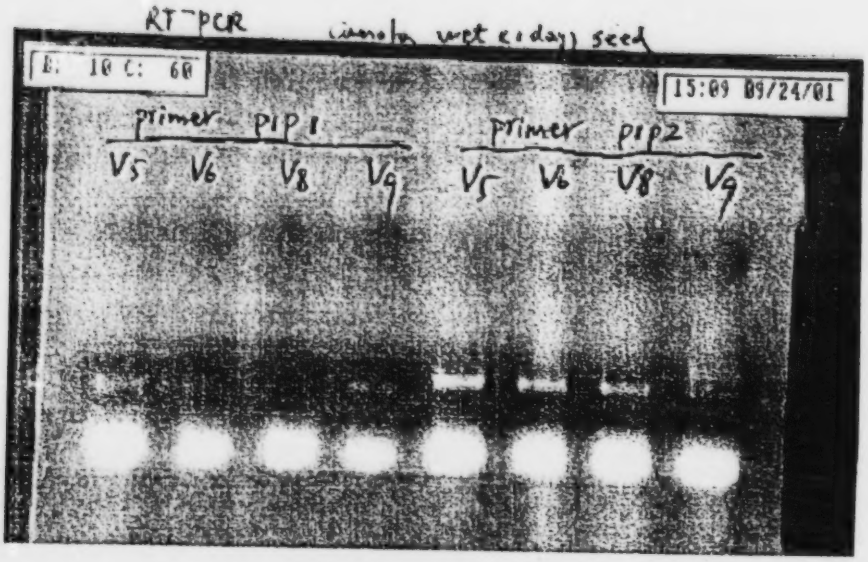

Figure 1. RT-PCR analysis of aquaporin gene expression from canola varieties of high and low germination vigour. V_5 and V_6 are varieties with high germination vigour and V_8 and V_9 are varieties with low germination vigour.

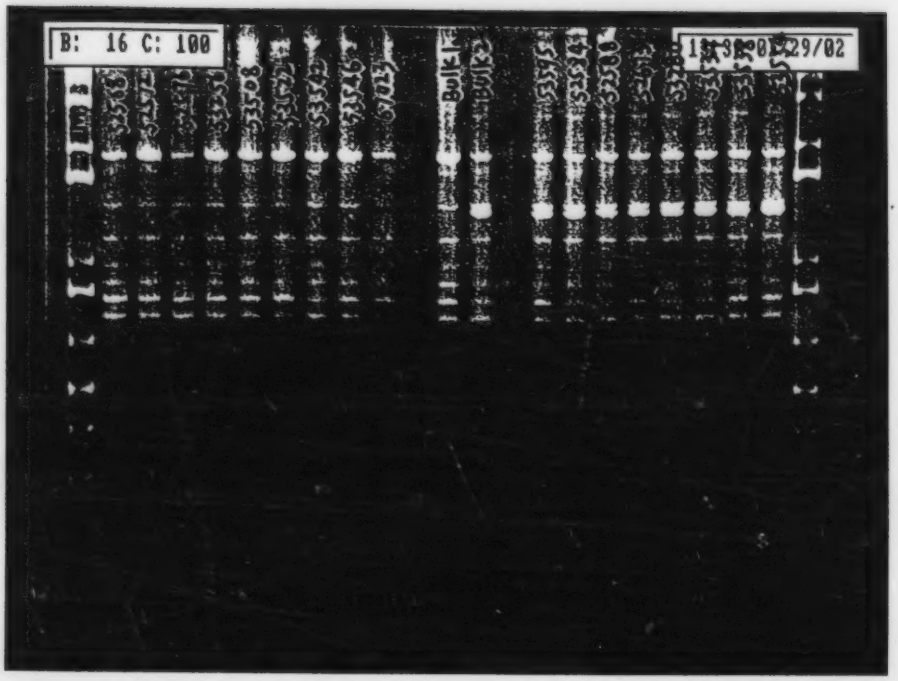

Figure 2. RAPD analysis using primer UBC317 to distinguish canola varieties for germination vigour. The two lines in the middle (Bulk 1 and Bulk 2) are from bulked DNA extracted from seed of low and high germination vigour respectively. The nine lines on the left represent varieties of low germination vigour while the lines on the right represent varieties of high germination vigour. The arrow indicates a band of 1300 bp that is highly correlated with high germination vigour.

(c) **Information of Benefit to Producers, Processors or Government**

Presentations

Development of a predictive selection technique to identify canola seed that loses germinability and seedling vigour during storage. Canola Vigour Steering Committee Meeting, Saskatoon Inn, November 1999, Saskatoon, Saskatchewan.

Seedling Vigour – Fact or Fiction. Canola Vigour Steering Committee, Saskatoon Inn, April 2000, Saskatoon, Saskatchewan.

Recent Developments in Selecting High Quality Canola Seed, 2001 Canola Industry Meeting, December 4, 2001, Sheraton Cavalier, Saskatoon, Saskatchewan.

Meetings and technical discussions with 20/20 Seed Labs, Inc., Nisku, Alberta and Grow Tec Inc., Nisku, Alberta.

(d) **Personnel**

Dr. G. Thompson – 18 months at half-time salary

Dr. X. Liu – 4 months at half-time salary

(e) **Equipment** – none purchased

(f) **Project Developed Materials** – none

(g) **Project Photos** – none

(h) **Acknowledgements** – All presentations included the ADF Logo indicating its support.

Development of Predictive Tests for Canola Seed Vigor

R.G. Thompson, R.H. Elliott and L.V. Gusta

The purpose of this project, initiated in March 1999, is to investigate aspects of canola seed quality that may form the basis of a predictive test for vigorous field performance. The need for vigor testing arises from the observation that some seed lots have good laboratory germination test results, but poor field performance. Even seed lots of the same variety, all having good germination characteristics when tested after harvest, may be affected differently by storage conditions, seed treatments and environmental factors, resulting in variable performance in the field.

Seed vigor is an extremely complex trait, affected by the interaction of numerous factors including seed quality, environmental conditions, seeding practices, pests and pathogens. In this study we are focussing on aspects of seed quality, using a selection of 18 canola seed lots that represent a wide range of germination and vigor characteristics. In initial experiments we have found a strong positive correlation between good germination in laboratory tests employing ABA and cold temperature, and rapid, uniform seedling emergence from soil.

Germination depends upon the uptake of water, but many conditions that impose stress on the seed, such as salinity, drought or temperature extremes, affect the availability of water. Water-channeling proteins, aquaporins, are induced during canola seed germination, and are reported to play an important role in regulating the permeability of cell membranes to water. Using canola seed lots that differ in vigor, we plan to investigate the expression of these genes with the goal of developing markers that correlate with high germination rates under field conditions. Such a test may discriminate between superior seed lots and those seed lots that are most likely to be adversely affected by stressful conditions. The test would also be useful in breeding programs for selection of the most vigorous lines.